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**EFFECTS OF NITROGEN SUPPLY ON
RATES OF PHOTOSYNTHESIS AND
RESPIRATION IN PLANTS**

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1935

By
KARL CLEMENS HAMNER

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CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 471

KARL C. HAMNER

Introduction

There is a conspicuous lack of recorded results of experiments to determine the effect of variations in the nutrient supply of plants on their photosynthetic and respiratory rates. SPOEHR and MCGEE (7), working with excised leaves of *Helianthus*, found that if the petioles were immersed in nutrient solutions containing certain amino acids the respiratory rate was increased. They also studied the photosynthetic rates of excised leaves after varying periods of exposure to darkness, and found some correlation between the respiratory rates and the photosynthetic rate. GREGORY and RICHARDS (3) grew barley plants with various deficiencies of nutrient supply along with others with complete nutrient solution. They measured the respiratory rates of single leaves removed from the plants at various stages in growth and found that nitrogen deficiency had little or no effect on the CO₂ assimilation rate and a slightly depressing effect on the respiratory rate. BRIGGS (1), using excised leaves, also studied the effect of mineral deficiencies on the assimilation rate.

The purpose of the work here described was to determine the rates of photosynthesis and respiration of leaves attached to the plant, and of whole plants grown under different conditions of nitrogen nutrition, when such plants were subjected to various changes in the relative planes of nutrient supply. For example, if plants had been grown for a period with very low nitrate supply, so that they became relatively high in reserve carbohydrates, their photosynthetic and respiratory rates were studied first under the low plane of nitrate supply, then they were shifted to a high plane of supply and these same rates continuously determined. Similarly, tests were

made of plants on a high plane of nitrate supply and then shifted to a low one, with various intermediate conditions, as seemed desirable at any given time.

I. Experimental work on tomato

METHODS AND APPARATUS, ETC.

Tomato plants were grown in the greenhouse in rich soil until they were about 6 inches high, at which time they were removed from the pots, the roots washed clean, and most of them clipped off close to the stem. The bases of the plants were then stuck 1-3 inches deep in clean moist quartz sand. New roots were soon developed. The nutrient solutions used throughout these experiments were the same as those described by NIGHTINGALE (6). The one containing nitrate was generally supplied for several days or longer following transplanting; but later all nitrates were omitted, and as a result the plants usually accumulated large reserves of storage carbohydrates as they continued to grow in the greenhouse.

At the beginning of each experiment six pots of plants were selected for uniformity and the relative vegetative or non-vegetative condition of the plants carefully noted. A general estimate of the reserves of nitrates and carbohydrates was determined by microchemical examination of the fresh tissue. The plants were then placed in a room which was held at constant humidity and temperature. Four 1000 watt tungsten bulbs placed at a distance of about 30 inches from the plants were used as the light source. An alternation of ten hours of exposure to light and fourteen hours in darkness was maintained throughout any given experiment.

For the detailed studies of carbon dioxide uptake and output, two leaves, one each from plants in separate pots, were selected. These will be referred to as the experimental leaves. They were green, approximately alike in appearance and area, and located as near the top of the plant as was possible, consistent with the ability to secure those which were fairly well matured and had attained practically full size. The leaf area was determined by printing an outline of the leaf on blueprint paper and later measuring the area by means of a planimeter. The light intensity was measured with a McBeth il-

luminometer. Adjustments were made so that the light intensity at the surface of each experimental leaf was the same at the beginning of each experiment.

The work with tomatoes was carried out in 1933. Since that time several changes have been made in the detail of the apparatus used. In its modified form it has been described by MITCHELL (4). The type of leaf chamber used, the method of sealing the plants into the chambers, the movement of gases through the chambers, and the methods of analysis of CO_2 are essentially the same as those described. In this early work, however, tungsten lamps were used as the light source. The concentration of CO_2 in the gas stream entering the leaf chamber was the same as in outside air. Under the light from tungsten bulbs there was a tendency for the leaf chambers to become heated, and in order to maintain the temperature inside the chamber equivalent to that outside it, the air in the chamber was cooled by passing it from the chamber by means of pumps (4) to coils in a cold water bath and then back to the chambers. There was no evidence in this work that the addition of nitrates to the plants used increased the rate of photosynthesis. This might be accounted for on the basis of some of the methods employed. The leaves inclosed in the gas chamber removed from 30 to 60 per cent of the CO_2 from the air stream passing over them and it may be that CO_2 was not present in sufficient quantities to permit an increase in the photosynthetic rate. It was also evident that the light source was not sufficiently intense nor of proper quality for growth and development of the plants, as shown by the fact that all the plants etiolated and lost some of their lower leaves. It was impossible to continue any given experiment for more than four or five days because the plants became so etiolated that they were no longer fit for use, or the leaves in the experimental chambers yellowed and died. It may be that if the proper light source were used and the CO_2 of the air stream in the chamber maintained at the same concentration as in atmospheric air, there would be an increase in the photosynthetic rate. Such a result was obtained, as is detailed later in the paper in the experiments on wheat.

EXPERIMENT I.—Six pots of plants were placed in the constant temperature room at 8 A.M. on July 28, 1933; two leaves were

selected on plants in different pots and sealed in the chambers. The circulation of gas through the chambers was started and the plants were illuminated until 4 P.M., which marked the close of the first light period. The plants were then in darkness for fourteen hours and this alternation of light and darkness continued throughout the experiment. Measurements of the CO_2 output of the experimental leaves were made throughout each period of darkness, records being taken at the end of every two hours. Nitrates were applied to three pots, including one of the pots with a plant bearing an experimental leaf, at the end of the second light period (in this case at 3 P.M., July 29).

The light intensity at the surface of each experimental leaf was 850 foot candles at the beginning of the experiment. At the end of the experiment it was 820 foot candles at the surface of the leaf on the plant which received no nitrate in the nutrient solution, and 850 foot candles at the surface of the other experimental leaf. The fall in light intensity in the former case was doubtless due to deterioration of the lamps used. The temperature of the room was 22°C . and the relative humidity was maintained at 75 per cent.

The plants when taken from the greenhouse were extremely high in reserve carbohydrates. All the cells of the mesophyll and palisade were filled with starch, which was abundant also throughout the parenchymatous tissues of the stem except at the very tip. The plants were hard and woody and showed an almost complete lack of nitrates. The changing composition of the plants was followed as closely as possible microchemically during the entire duration of the experiment. Those plants which were not inclosed in the small chamber and to which the nitrates were added remained high in carbohydrates, although they became slightly etiolated. The upper internodes lengthened, and growth at the tip took place rapidly. Yellowing and death of the lower leaves also occurred. The leaf inclosed in the chamber showed progressive yellowing from the margin of the leaflets inward during the course of the experiment and at the close was about to fall from the plant.

Those plants to which nitrates were added, when compared with those which received no nitrogen, appeared slightly greener, and both stem and leaves enlarged more than in the case of the minus

nitrogen plants. These also etiolated somewhat and lost their lower leaves. In four to six hours after nitrates had been added tests for nitrates were positive well up in the plants, and in twenty-four hours nitrates were in abundance throughout. Nitrites became apparent about two hours after the nitrates were detectable. After fourteen hours the plus nitrate leaf was noticeably greener than the minus nitrogen one, and had enlarged appreciably.

TABLE I
EXPERIMENT I: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO ₂ RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)			
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD	4TH DARK PERIOD
PLUS NITRATE				
1.	167	84	171	
2.	119	84	163	143
3.	116	92	163	86
4.		95		86
5.	99		155	86
6.	73	109	131	73
MINUS NITRATE				
1.	203	120	121	
2.	155	104	112	73
3.	131	112	118	86
4.	129	99	111	86
5.	114	94	104	86
6.	114	87	104	87

The results of the respiratory measurements are given in table I. It is obvious that following the addition of nitrates, the respiration rate is appreciably increased.

EXPERIMENT II.—This experiment was begun in the constant temperature room on August 2, 1933. The same details of procedure were followed as before. When placed in the room the plants were nitrate-free but were slightly lower in reserve carbohydrates than those of the previous lot. They appeared slightly greener and the

stem tips were softer. They were high in reserve carbohydrate, however, and behaved very similarly to those of experiment I. During the course of the experiment they etiolated somewhat and lost their lower leaves. The experimental leaf of the plant to which no nitrates had been added became yellow as before and was ready to fall from the plant at the close of the experiment. Those plants to which

TABLE II
EXPERIMENT II: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO ₂ RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)		
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD
PLUS NITRATE			
1.....	202	144	199
2.....	148	125	165
3.....	148	114	165
4.....	119	119	153
5.....	125	94	165
6.....	114	95	153
MINUS NITRATE			
1.....	254	188	126
2.....	151	126	108
3.....	126	126	100
4.....	126	107	108
5.....	125	115	100
6.....	123	126	111

nitrates were added became noticeably greener and enlarged considerably.

Nitrates in slight amounts made their appearance in the upper parts of the plants in twelve hours after their application. Nitrites were evident several hours after the nitrates appeared in the tops, but were present only in very small amounts.

The results of the respiratory measurements are given in table II. In general the plants responded more slowly to nitrate application

than did the plants of the previous experiment. As compared with the results of experiment I, there was less increase in the rate of respiration after the application of nitrates and the response was delayed for a longer period.

EXPERIMENT III.—The plants were removed to the constant temperature room on August 8, 1933 at 8 A.M. and the measurements

TABLE III
EXPERIMENT III: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO ₂ RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)		
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD
PLUS NITRATE			
1.....	182		200
2.....	133	137	168
3.....	139	128	159
4.....	139	111	168
5.....	135	106	166
6.....	116	90	164
MINUS NITRATE			
1.....	190	143	
2.....	151	136	78
3.....	147	133	102
4.....	149	88	89
5.....	147		71
6.....	125	100	

started at 4 P.M. as before. The procedure followed was the same as that used in the previous two experiments. The plants used might be described as being intermediate in condition and response between the plants of the first and the second experiments. They were slightly taller than those of the previous two lots, but the number and size of the leaves were about the same. They were not so high in reserve carbohydrates as the first lot of plants, but higher than the second. Upon the application of nitrates the plants turned

greener and enlarged as before. Nitrates were present in small amounts after six to eight hours, and nitrites appeared a few hours later.

The results of the respiratory measurements are given in table III. In general the results occupy an intermediate position between those obtained in the first and second experiments.

TABLE IV
EXPERIMENT IV: RESPIRATION IN TOMATO PLANT

INTERVAL OF READ- ING (EACH OF 2 HOURS' DURATION)	CO ₂ RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)		
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD
	PLUS NITRATE		
1.....	63		162
2.....	60	64	173
3.....	55	68	184
4.....	48	64	
5.....	52	75	160
6.....	32	72	139
	MINUS NITRATE		
1.....	81		18
2.....	81	37	18
3.....	67	24	10
4.....	67	29	
5.....	70	32	10
6.....	56	36	0

EXPERIMENT IV.—Except for the fact that they were slightly larger, contained more lignified tissue, and had a slightly hollow stem, the plants used in this experiment were similar to those used in the first experiment. They were extremely high in reserve carbohydrates; and as in the first experiment, all of the mesophyll and palisade cells of the leaves were filled with starch which was abundant also throughout the parenchymatous tissues of the stem except at the very tip. No reserves of nitrates were apparent.

The plants were placed in the constant temperature room on August 15, 1933 and treated as usual. Nitrates were present throughout the plants within three hours after their application in the nutrient solution, and nitrites appeared two hours later. The leaves of the plants to which nitrates were added became greener than the others within eight to ten hours after the application of nitrates.

Results of the gas analyses are given in table IV. The results are similar to those in experiment I except that the increase in rate of respiration is greater.

SUMMARY

On the basis of the foregoing data it is evident that the composition of any given plant is in a marked degree correlated with its respiratory activity. Thus tomato plants very high in carbohydrates but low in their nitrate or soluble nitrogen content may have a relatively low respiratory rate, whereas one which is higher in its nitrate content will have an appreciably higher one. Or any given plant high in its carbohydrate reserves will have its respiratory rate increased if nitrates are supplied to it in abundance, other conditions remaining the same. Although all the plants used in the various experiments were very high in reserve carbohydrates, a relatively slight change in the amount of this reserve had a great effect upon the time required for nitrates to make their appearance in the tops of the plants after they had been applied in the nutrient solution. In every case the addition of nitrates to plants high in reserve carbohydrates caused an increase in respiration, the greater such reserve the more nearly immediate the response and the greater in degree. In the case of plants extremely high in reserve carbohydrates the response was almost immediate and very marked. In the first and last experiments respiration of the plants to which nitrates had been added increased to three times that of the plant which received no nitrate. In the case of plants slightly lower in reserve carbohydrates the response was not so great, nor did it occur at once. In the second experiment a response was noticeable only after twenty-four hours, and the increase in respiration was about 100 per cent. In the third experiment the response became evident in twenty-four hours, and the total increase was 120 per cent. In each case the increase in rate

of respiration seemed to take place subsequent to the appearance of nitrates in the top of the plant; in no instance did it occur prior to such appearance, whatever the length of time elapsing after their application in the nutrient solution.

II. Experimental work on wheat

It was desired to supplement the work on tomatoes, since the measurements were made on single leaves instead of on entire plants. Also it was apparent that the plants would not accumulate reserves of carbohydrates nor maintain those reserves already present when they were placed under Mazda lights. In the work on wheat it was found possible under artificial illumination to grow plants which would continue to increase in size and in dry weight. By varying the nitrogen supply in the nutrient solution, plants either very high or very low in reserve carbohydrates, as was desired at any particular time, were obtainable. It was possible also to obtain separate quantitative measurements on the respiration of roots and of tops and also measurements of the CO_2 assimilation of the entire tops.

The apparatus finally devised and used in this work is that described in detail by MITCHELL (4). That part of the apparatus described for experimentation with entire plants was designed for these experiments.

Wheat grains of the Marquis variety, secured from Corvallis, Oregon, were sterilized in 0.25 per cent uspulun for five minutes and planted in sterilized quartz sand. In each experiment 50 grains were planted per pot. Six-inch clay pots and pyrex beakers of 1500 cc. capacity and with necessary outlets were used as containers. These were placed in the constant temperature room, watered with distilled water for the first week, and exposed to a light intensity of about 300 foot candles. At the end of the first week the pots containing the plants were placed in a pan of distilled water so that the water level reached the upper surface of the sand in the pot, and a little of the sand around the base of the seedlings was washed away. The ungerminated grains were picked out. All seedlings in excess of fifteen per pot were removed in entirety, care being taken that those remaining be as uniform in size as possible.

The pots were then placed about 30 inches from the light in posi-

tions such that the plants in each pot were exposed to an intensity of about 800 foot candles for a period of twelve hours. The light was then turned off and the plants were in darkness for the remaining twelve hours of each day.

The nutrient solutions employed throughout these experiments were the same as those used by NIGHTINGALE (6). One of these contained no nitrate, the other contained nitrate in abundance. For four days following the time when the cultures were placed in full light they were supplied with the nutrient solution containing no nitrate, diluted one-half with distilled water. For at least two days more all the cultures received the same solution, undiluted. Subsequent treatment of the plants varied in the several experiments conducted. In all of them, however, the plants were sealed in the chambers at twelve to fourteen days after the sowing of the grains. After sealing in the chambers all the cultures were supplied with minus nitrate solution for two days more. Then, commencing at the end of the light period of the third day, half of the plants received the same minus nitrate solution and half the plus nitrate solution. This treatment was continued throughout any given experiment.

During the first two days in the chambers, readings were made of the CO_2 uptake during the last four hours of the light period, and CO_2 output during the first four hours of the dark period. At the end of the light period of the third day the plus nitrate nutrient solution was applied to one-half of the cultures, and readings on CO_2 output of both the plus and minus nitrate cultures made for the following four-hour dark period. Subsequent readings of uptake and output were made for such periods and at such times as already stated for the first two days the plants were in the chambers.

It was found that essentially the same values were obtained for the uptake and output over a given time interval, whether the readings were made at the beginning or the end of a given light or dark period. For convenience in making measurements and in recording results, only readings for the last four hours of the light period and the first four hours of the dark period of each day are given.

In each experiment thirty groups of plants were used. Six of these were the pyrex beakers mentioned, and twenty-four were the 6-inch clay pots. The plants in the six beakers were used for the measure-

ments of CO₂ uptake and output. At the time when nitrates were added to some of the plants, those in eight of the clay pots were harvested and constituted the initial sample. Such plants, of course, had received no nitrate. Eight of the remaining clay pots and three

TABLE V
EXPERIMENT V: QUANTITATIVE DETERMINATIONS OF CO₂ UPTAKE
AND OUTPUT BY YOUNG WHEAT PLANTS

AGE OF CULTURE IN DAYS	CO ₂ ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EX- POSURE TO LIGHT (MG.)	CO ₂ LIBERATED PER 100 PLANTS DURING 4-HOUR IN- TERVAL OF PERIOD IN DARKNESS (MG.)		CO ₂ ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EX- POSURE TO LIGHT (MG.)	CO ₂ LIBERATED PER 100 PLANTS DURING 4-HOUR IN- TERVAL OF PERIOD IN DARKNESS (MG.)	
		ROOTS	TOPS		ROOTS	TOPS
		RECEIVING NO NITROGEN IN NUTRIENT SOLUTION				
14.....	186	32.4	15.2	164	33.2	11.9
15.....	186	29.8	15.5	152	30.6	14.9
16.....	186			154		
AFTER ADDITION OF NITRATES TO NUTRIENT SOLUTION SUPPLIED TO SOME OF THEM						
	PLUS NITRATE			MINUS NITRATE		
16.....		41.7	15.5		29.8	13.6
17.....	221	42.1	24.2	133	29.6	13.7
18.....	290	35.6	18.0	140	30.0	14.0
19.....		21.0	15.2		29.8	14.1
20.....	310	31.9	21.3	154		
21.....	290			140	30.8	11.1
22.....	279	35.1	21.5	133		
23.....	279			128	31.9	10.4
24.....		39.0	22.3			
25.....	234			138	30.1	8.9
26.....	234	41.0	23.8	140		
27.....					30.4	9.1
28.....	226	42.8	29.0	168	29.3	8.7

of the beakers were then supplied with nitrates in the nutrient solution and the others were continued with the minus nitrogen nutrient solution. At the end of the experiment all the plants in the clay pots were harvested and constituted the final sample.

DISCUSSION OF RESULTS

The results of three separate experiments are given here, since they are representative of several which show the same results. The first experiment extended from the time the plants were fourteen days old until they were twenty-eight days old. At the age of fourteen days none of the plants showed definite visible signs of nitrogen deficiency except that they were a light green. At the end of the experiment, however, those plants which had been supplied with no nitrogen in the nutrient solution showed definite injury. The lower leaf was usually yellow throughout its length and nearly dead; often the second leaf was dead at the tip and yellow nearly half the dis-

TABLE VI
EXPERIMENT V

	DRY WEIGHT PER 100 PLANTS (GM.)			FRESH WEIGHT PER 100 PLANTS (GM.)		LEAF AREA PER 100 PLANTS (SQ. DM.)
	ROOT	TOP	TOTAL	ROOTS	TOPS	
Initial sample.....	1.400	1.627	3.027	20.90	12.1	5.00
Plus nitrate plants 14 days later.....	1.803	4.352	6.155	37.1	34.9	13.06
Minus nitrate plants 14 days later.....	2.430	2.168	4.598	47.5	16.4	6.04

tance toward the base of the leaf. The rest of this leaf and the rest of the leaves were a yellowish green in color. Those plants to which nitrates had been added were a deep green except for the lowest leaf which often was partially dead. The other leaves were a fresh green color and continued to increase rapidly in size, more than doubling in area by the end of the experiment. The significant data are given in tables V-VI.

A second experiment of the same general nature as the first was also performed. All conditions were kept as nearly the same as possible, except that readings on uptake and output were begun when the plants were twelve days old instead of fourteen. This was in order that the plants might not have undergone as much injury from nitrogen deficiency as under the first experiment. The data are given in tables VII-VIII.

A third experiment was carried out to eliminate some of the variations apparent in the preceding two experiments and to obtain data concerning the changes in root volume and root area. The root volume was obtained by placing the clean fresh roots in water and

TABLE VII
EXPERIMENT VI: QUANTITATIVE DETERMINATIONS OF CO₂
UPTAKE AND OUTPUT BY YOUNG WHEAT PLANTS

AGE OF CULTURE IN DAYS	CO ₂ ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EX- POSURE TO LIGHT (MG.)	CO ₂ LIBERATED PER 100 PLANTS DURING 4-HOUR IN- TERVAL OF PERIOD IN DARKNESS (MG.)		CO ₂ ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EX- POSURE TO LIGHT (MG.)	CO ₂ LIBERATED PER 100 PLANTS DURING 4-HOUR IN- TERVAL OF PERIOD IN DARKNESS (MG.)	
		ROOTS	TOPS		ROOTS	TOPS
	RECEIVING NO NITROGEN IN NUTRIENT SOLUTION					
12.....	178	32.5	20.5	178	31.9	14.6
13.....	189			178		
	AFTER ADDITION OF NITRATES TO NUTRIENT SOLUTION SUPPLIED TO SOME OF THEM					
	PLUS NITRATE			MINUS NITRATE		
13.....		51.9	17.5		31.9	14.9
14.....	201	40.4	17.3	165	33.5	14.3
15.....	247	32.4	19.1	161	34.6	13.3
16.....	258	34.6	20.5	149		
17.....	266	38.6	24.5	160	31.4	14.1
18.....					32.4	12.8
19.....	279	38.6	25.2	146		
20.....		41.2	26.8		31.9	12.2
21.....	247			160		
22.....	237	45.2	31.1	145	31.9	12.8
23.....						
24.....	235	47.9	31.9	142	30.6	8.5

measuring the volume of the displaced liquid. The root area was an approximation calculated from the area covered by the projection of the root system on to a plane surface. The latter area was obtained with an apparatus described by MITCHELL (5). The clean fresh roots were spread out on a plate glass and the area covered

by their shadows measured as described. This area was multiplied by 3.14 to give root area. All conditions were kept as nearly the same as those of the first experiment as possible, except that the plants were watered with minus nitrogen nutrient solution diluted one to ten with distilled water during the first week. Screens were placed behind the chambers in such a manner that some of the light passing through or around them might be reflected back, thus giving a more

TABLE VIII
EXPERIMENT VI

	DRY WEIGHT PER 100 PLANTS (GM.)			FRESH WEIGHT PER 100 PLANTS (GM.)		LEAF AREA PER 100 PLANTS (SQ. DM.)
	ROOT	TOP	TOTAL	ROOTS	TOPS	
Initial sample	1.160	1.488	2.648	20.8	11.66	4.47
Plus nitrate plants 12 days later	1.380	3.540	4.920	35.0	31.8	10.82
Minus nitrate plants 12 days later	2.00	2.04	4.04	45.0	15.8	4.86

nearly uniform lighting of the entire chamber and of the plants inside it. The significant data are given in tables IX and X.

It will be obvious at once that there was some fluctuation in the readings, each of which is the average of three separate pots of plants (45 plants in all), but there are definite and marked trends, which are evident in all the experiments. It is not yet possible to account precisely for some of the variations in CO₂ uptake and output. They may have been due to minor fluctuations in the relative humidity or to other seemingly minor variations in environmental conditions. Great care was taken to keep these factors as nearly uniform as possible, but at times they were not absolutely constant. As already stated, screens were placed behind the plants in the last experiment in order to obtain a more nearly uniform lighting of the entire chamber and of the plants. This was done because in the previous experiments a slight fog had accumulated on one side of the chambers and it was thought that this might reflect varying amounts of light according to its density. The readings in the last experiment are slightly more uniform than those of the previous

experiments. All the experiments agree in their general trends and the trends shown are definite and of sufficient magnitude to remain unobscured and valid in spite of the variations shown.

TABLE IX
EXPERIMENT VII: QUANTITATIVE DETERMINATIONS OF CO₂ UPTAKE AND OUTPUT BY YOUNG WHEAT PLANTS

AGE OF CULTURE IN DAYS	CO ₂ ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EX- POSURE TO LIGHT (MG.)	CO ₂ LIBERATED PER 100 PLANTS DURING 4-HOUR IN- TERVAL OF PERIOD IN DARKNESS (MG.)		CO ₂ ASSIMILATED PER 100 PLANTS DURING 4-HOUR INTERVAL OF PERIOD OF EX- POSURE TO LIGHT (MG.)	CO ₂ LIBERATED PER 100 PLANTS DURING 4-HOUR IN- TERVAL OF PERIOD IN DARKNESS (MG.)	
		ROOTS	TOPS		ROOTS	TOPS
		RECEIVING NO NITROGEN IN NUTRIENT SOLUTION				
14.....	167	29.6	15.1	173	30.9	14.0
15.....	175	29.0	14.7	172	29.8	13.8
16.....	178			168		
AFTER ADDITION OF NITRATES TO NUTRIENT SOLUTION SUPPLIED TO SOME OF THEM						
PLUS NITRATE				MINUS NITRATE		
16.....		40.0	16.1		29.6	13.5
17.....	219	45.0	16.6	170	30.1	14.1
18.....	220	30.9	15.8	166	30.0	13.7
19.....	260	32.1	17.1	166	31.2	13.3
20.....	280	34.2	17.0	176	30.8	13.5
21.....	250	34.0	18.6	174	29.9	12.8
22.....	245	36.0	20.5	168	30.4	13.1
23.....	260	36.1	23.9	176	29.8	12.5
24.....	260	37.8	25.0	168	29.8	11.8
25.....	262	39.1	25.0	176	30.7	10.8
26.....		41.6	26.2		30.6	10.6
27.....	256	42.7	26.9	167	31.2	10.1
28.....	250	45.3	28.7	170	30.5	9.2
29.....	245	50.8	30.9	172	30.2	8.7
30.....	238			174		
31.....	240	52.9	32.4	170	31.8	8.8

CULTURES TO WHICH NO NITRATE WAS SUPPLIED.—Perhaps the most striking result is the fact that the minus nitrate plants, although part of the leaves died and those that remained alive became

yellow to yellowish green and increased but little in size, yet were able to assimilate relatively large quantities of CO_2 . That there is not an error of measurement involved is confirmed by the figures on the dry weight of these plants. These showed an increase comparable with the amount of CO_2 absorbed over the amount lost in respiration. This must mean, therefore, that on the basis of unit area of the leaf the rate of carbon fixation has undergone practically no decrease during the experiment. It is not possible to say with precision just how many living active cells capable of photosynthesis there may

TABLE X
EXPERIMENT VII

	DRY WEIGHT PER 100 PLANTS (GM.)			FRESH WEIGHT PER 100 PLANTS (GM.)		LEAF AREA PER 100 PLANTS (SQ. DM.)	ROOT AREA PER 100 PLANTS (SQ. DM.)	ROOT VOL- UME PER 100 PLANTS (CC.)
	ROOT	TOP	TOTAL	ROOTS	TOPS			
Initial sample . .	1.20	1.60	2.80	20.5	12.05	4.17	12.62	27.7
Plus NO_3 plants 16 days later . .	1.87	5.28	7.15	36.3	45.32	14.77	19.77	40.1
Minus nitrate plants 16 days later	2.40	2.48	4.88	46.04	15.5	4.51	21.54	34.5

have been nor what their exact volume was, but that those which were capable of functioning continued to do so at a fairly rapid rate there is no doubt. The relatively large capacity of these cells to fix the quantity which they did may well furnish a definite basis for understanding why plants which are small because of nitrogen starvation are still able to accumulate large amounts of carbohydrate or carbohydrate derivatives, either as reserve foods or structural materials. It may also aid in an interpretation of the behavior of some species when subjected to short photoperiods.

The total amount of CO_2 output of the tops during the periods of the experiment showed a quantitative decrease. This is in accord with the results of GREGORY and RICHARDS (3). Thus the rate of respiration per unit of area would at first seem to be markedly less, but actually may not have been so, since some of the apparently liv-

ing tissues measured may actually have been dead. Again the results are of such nature that valid deductions are possible.

In contrast to the relatively small increase in the tops of these minus nitrate plants, the roots gained appreciably in volume, area, and more than 100 per cent in dry weight. Since the total amount of CO_2 given off remained about the same despite the increase in volume of the root and since there was virtually no dead tissue present, the respiratory rate showed an actual decrease. It is apparent, therefore, that the elaborated materials from the tops were in this case used in extension of the root system and the building up of a dry weight reserve. This is in marked contrast to the roots from the plus nitrate cultures, as will be discussed later.

CULTURES TO WHICH NITRATE WAS SUPPLIED.—The tops of these plants began to increase markedly in size after the application of nitrate. They were a fresh bright green and by the end of the experiment had more than doubled in dry weight. The volume of CO_2 absorbed increased markedly during the first five days, then declined somewhat despite the continued increase in leaf area, and finally leveled off to an amount slightly in excess of that before the nitrate was applied. Thus it is obvious that the rate of photosynthesis per unit of leaf area at first markedly increased and then later actually decreased. It was thought that most of the leaves which expanded during the experiment might already have been differentiated when nitrate was added so that there was little or no increase in the number of stomata operative. That this was not true was shown by stomatal counts. It may be that this decrease in the rate of CO_2 uptake is associated with the increasing age of the plants. GREGORY and RICHARDS (3) claim that in barley there is a decrease in the photosynthetic rate of all of the leaves as the plant grows older. There was no such decrease in the minus nitrate plants, however. It is possible that at the beginning of the period there was a metabolizable carbohydrate reserve in the leaves which soon became used up in expansion of the volume of leaf and that the newer chemical relations set up within the leaf were not favorable to increased photosynthesis. There was at all times an abundance of CO_2 in the surrounding atmosphere so that it did not become a limiting factor. The results, which were obtained repeatedly in these experi-

ments, were not wholly in accord with what might have been expected on the basis of many other results already recorded in the literature. This difference, however, makes the results obtained all the more interesting, for they certainly call for a further chemical investigation of leaves in relation to their capacity for CO_2 uptake and fixation.

In general the tops of the plants showed a gradual rise in CO_2 output as the leaves expanded. Except in one instance, there was no abrupt rise and fall, and in that instance there was a high initial carbohydrate content of the leaves. On the basis of unit of leaf area, the respiration rate remained about the same or even slightly decreased, despite the increased expansion rate of the leaves, which might have been expected to be accompanied by a considerably increased respiration rate. It seems obvious that most of the products of photosynthesis were used in the manufacture of the tissues of the leaf, as reflected in the increased dry weight.

In contrast to the roots of the minus nitrate cultures, those of the plus nitrate cultures gained little in dry weight over the initial cultures. This is in harmony with many findings relative to root-top ratios when plants are liberally supplied with nitrates, and especially if reserve carbohydrates are small in quantity or conditions for their synthesis or translocation limited. It is interesting to note that the volume of the root system of these cultures (table X) increased appreciably and more in relation to root area than did the minus nitrate cultures. This might be expected since the individual roots were much greater in diameter and more succulent than those of the minus nitrate plants. After the application of nitrates the CO_2 output in these experiments rose sharply and increased markedly, such increase being followed presently by an abrupt drop to an amount about equal to that of the control plants, and then another slight increase. Since there was some slight increase in the volume of the roots during the experiment, the rate of respiration per unit of volume at the end of the experiment was about equal to that of the initial plants.

Recalling the fact that the roots of the minus nitrate plants, although showing a marked increase in their carbohydrate content, did not show a marked increase in respiration rate, it is safe to say

that the presence of carbohydrates alone in and of themselves do not necessarily mean an increased respiration rate. But if nitrates are added to the nutrient solution, then it is possible that these may become elaborated into amino acids, proteins, and the like, which may have a marked effect on the respiration rate, as SPOEHR's results have previously shown. Thus there could readily be a rapid rise of respiration rate in the case of the roots in these experiments. Similarly such a rise might be expected to be followed by a fall unless the reserve of carbohydrates were very large or unless there was a comparatively large amount of carbohydrates constantly being translocated to the roots. This latter condition apparently did not prevail, such carbohydrates as were synthesized being utilized largely in top expansion. Finally, although nitrates were abundant even after the first brief period, the carbohydrate supply in the roots may well have become a limiting factor in the respiratory rate.

A condition very similar to that shown by these plus nitrate roots was clearly indicated by the tomato leaves, which contained a large supply of carbohydrates at the time of application of the nitrates, and also by the tops in one of the experiments with wheat, when such tops contained a fairly large initial content of carbohydrates. The abrupt rise of respiration rate was not observed in the tops of those plants which did not have such a reserve, even though nitrates were added, as has already been stated.

Comparison of work on tomato and wheat

With respect to respiration, the same general results were secured with both plants. There can be no doubt concerning the resultant marked increase in the respiration rate by the application of nitrate to the plant provided there is an initial carbohydrate reserve. The rate is roughly proportional to the amount of such carbohydrate content. This is true whether the organ experimented upon is leaf or root.

It is equally clear that a relatively high photosynthetic rate may be maintained by leaves low in soluble forms of nitrogen, relatively low in content of chlorophyll, and relatively high in carbohydrate content of the cells. These findings should be accompanied by critical qualitative and quantitative chemical analyses at the time

the demonstrations are made. Detailed analyses are not available for the experiments presented, but roughly quantitative measurements of some of the compounds were made, and have already been commented upon.

The apparatus as herein described is adequate for the determination of CO_2 output and uptake by plants. It is delicate and requires care in its operation, but the results obtained with it under uniform conditions may be relied upon. The next step is to correlate these findings with the chemical composition of the organs being studied and with critical quantitative measurements of areas, volumes, and the like, so that the rates of the processes of photosynthesis and respiration may be precisely determined. This is particularly necessary because the present results, found over and over again in these experiments, are not completely in accord with some previous results as found in the literature and more or less taken for granted as standard and final. The whole subject is still open for further critical experimentation and a revaluation of the data already recorded.

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